Measurement of the concentrations of raw material, soya oil and products, mannosyl erythritol lipid, in the fermentation process using near-infrared spectroscopy

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Introduction

Nowadays, chemical synthesised surfactant is used in many areas such as detergents, emulsifiers, dispersing agents, coagulant agents, wetting agents, foaming agents, defoaming agents, lubricants, softening agents, microbicides, etc. Although the production cost of chemical synthesised surfactant is low, it has several problems, including high toxicity, low bio-degradability and heavy environmental pollution. However, now the production of biological synthesised surfactant is being studied and developed. Biosurfactant has several advantages, low or harmless toxicity, high bio-degradability and light environmental pollution, although the production cost of it is higher than that of chemical synthesised surfactant.

Yeast, *Kurtzumanomyces* sp. I-11 produces mannosyl erythritol lipid (MEL) from soya oil. Soya oil is classified by triglycerides and is composed of 53% of linoleic acid, 23% of oleic acid, 11% of palmitic acid and 8% of linolenic acid as fatty acid moieties. MEL is the typical amphiphilic compound including both lipophilic and hydrophilic moieties in the molecule and is composed of mannose, erythritol and fatty acids (Figure 1).

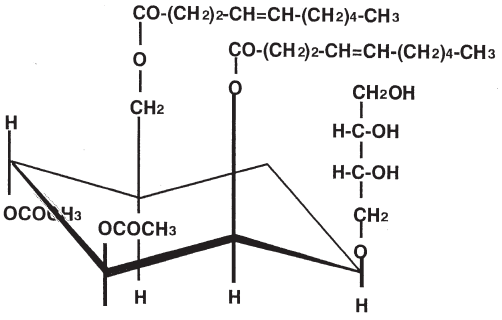

Figure 1. Molecular structure of mannosyl erythritol lipid (MEL).

In the fermentation process, measurement and control of the concentrations of raw materi-

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als and products in the culture broth are very important to obtain high productivity and low production cost. Initially, direct measurement of the concentrations of soya oil and MEL in the culture broth were studied. However, no good results have been obtained yet. First, the culture broth was nonhomogeneous. It consisted of air bubbles, water, soya oil, MEL and microorganism cells. It was difficult to make the homogeneous culture broth although the culture broth was mixed at high speed. Second, optical density of the culture broth became high as fermentation developed. As a result, the output of the near infrared (NIR) sensor was saturated. Third, soya oil in the culture broth attached on the inner wall of the cuvette of the spectrophotometer. So it was difficult to obtain the accurate values of the absorbance of the culture broth. However, it will be possible to solve the problem by intermittently washing with organic solvents such as ethyl acetate and acetone.

In this study, soya oil and MEL were extracted from the culture broth with ethyl acetate and the measurement system for the concentrations of soya oil and MEL in the ethyl acetate extract is developed using NIR spectroscopy.

Materials and methods

Microorganism and culture conditions

Five hundred mL of the seed culture of *Kurtzumanomyces* sp. I-11 was added into a 7 L of production medium in a 10 L fermentor (BMS-10PII, Able Co., Shinjuku-ku, Tokyo, Japan). The composition of the production medium contained the following components (gL^{-1}): soya oil variable; NH_4NO_3 0.5; KH_2PO_4 0.4; $MgSO_4 \cdot 7H_2O$ 0.2; yeast extract 1. To measure the concentrations of soya oil and MEL, about 100 mL of the culture broth was drawn out during several runs of the fermentation.

NIR spectroscopy

Four mL of ethyl acetate was added to 4 mL of the culture broth and mixed well. The extraction procedure was repeated three times and the ethyl acetate layer was transferred and combined in a mess flask.The ethyl acetate extract was injected into a cuvette with a 2 mm light path length. After putting the cuvette in the cell holder of an NIR spectrophotometer (NIRS6500SPL, Nireco Co., Hachioji, Tokyo), the absorbance from 400 to 2500 nm was measured at 2 nm intervals. The values of segment and gap size of the calculation of second derivative spectra were 20 and 0 nm, respectively.

To produce calibration equations, multiple linear regression (MLR) using the least-squares method was carried out between the NIR spectral data and the concentrations obtained by the conventional methods, C_{act}, for the calibration sample set.

To select a wavelength used for calibration equation, NIR spectra of MEL, soya oil, mannose, erythritol, ethyl acetate, ethyl ether, 1-butanol and butyric acid were measured. The MEL, soya oil, ethyl acetate, ethyl ether, 1-butanol and butyric acid were injected into the cuvette with a 2 mm light path length and NIR transmittance spectra were measured. The mannose and erythritol powder were packaged in a standard sample cup (Nireco Co.) and NIR reflectance spectra were measured.

Conventional analysis

The concentrations of MEL and soya oil in the ethyl acetate extract of the culture broth were measured using a thin layer chromatography with a flame-ionisation detector (TLC/FID) (Iatroscan newMK-5, Iatron Laboratories Inc., Tokyo, Japan). Microbial cell concentration in the culture broth was measured using the oven drying method.

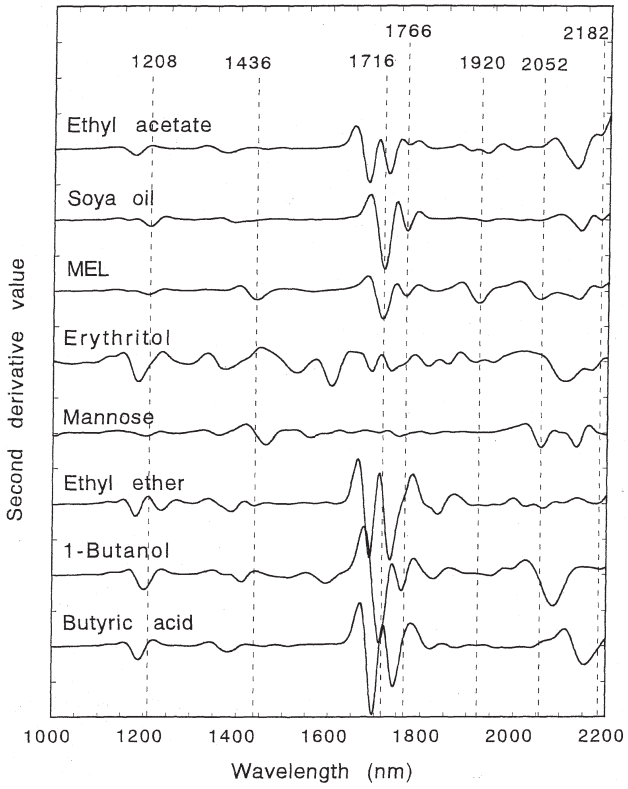

Figure 2. Second derivative spectra of authentic compounds.

Results and discussion

Second derivative NIR spectra of MEL and soya oil

Second derivative NIR spectra of ethyl acetate, MEL, soya oil, mannose, erythritol, ethyl acetate, ethyl ether, 1-butanol and butyric acid are shown in Figure 2. The three significant negative peaks were observed at the wavelengths of 1436, 1920 and 2052 nm on the second derivative NIR spectrum of MEL. The peak at 2052 nm may be caused by a mannose moiety of MEL because the NIR spectrum of mannose powder had a sharp peak at 2055 nm. The peak at around 1436 nm may be caused by ether linkage, C–O–C. Five ether linkages are in the MEL as shown in Figure 1. The peak at around 1436 nm was observed on the spectrum of ethyl ether while the peak was not observed on either spectra of 1-butanol and butyric acid. The peak at around 1920 nm may be caused by a combination band of O–H stretching and O–H deformation.[1] Therefore, the peak at around 1436 or 2050 nm was selected as the first wavelength for MEL calibration.

Four significant negative peaks were observed at 1208, 1716, 1766 and 2182 nm on the spectrum of soya oil and the absorption at these wavelengths was stronger than that of MEL. These negative peaks were mainly caused by the fatty acid moiety. It has been reported that the absorption band at around 1208 nm could be assigned to the second overtone of C–H.[1] The absorption at around 1716 nm

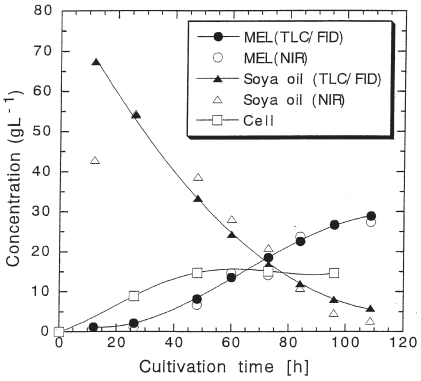

Figure 3. Time courses of the concentrations of soya oil, MEL and microbial cell.

and 1766 nm could be assigned to the first overtone of C–H.[1,2] The absorption at around 2182 nm could be assigned to the combination band of C–H with C=C.[2,3] Therefore, the peak at around 1766 or 2180 nm was selected as the first wavelength for soya oil calibration and the influence of MEL was corrected by using the second wavelength.

Calibration and validation for determining soya oil and MEL in ethyl acetate extract of the culture broth

MLR was conducted on the second derivative spectra and C_{act} for soya oil in ethyl acetate extract to formulate a calibration equation. The following calibration equation of soya oil was obtained.

$$C_{pre,soya\ oil} = -266 - 3680 \cdot A_1 + 2414 \cdot A_2 \quad (1)$$

Here, A_1 and A_2 are the values of second derivative spectra at 2178 and 2090 nm, respectively. The values of regression coefficient (R) and the standard error of calibration (SEC) were 0.974 and 0.77 gL^{-1}, respectively.

Validation of the calibration equation, Equation 1, was carried out. The soya oil concentration in the prediction sample set ($n = 54$) was predicted using Equation 1 and compared with the values of C_{act}. The standard error of prediction (SEP) was 0.56 gL^{-1}. Good agreement between C_{act} and C_{pre} was observed with a correlation coefficient (r) of 0.979.

For the MEL calibration, the following equation was obtained:

$$C_{pre,MEL} = -56 - 5769 \cdot A_1 + 8993 \cdot A_2 \quad (2)$$

Here, A_1 and A_2 are the values of second derivative spectra at 2040 and 1312 nm, respectively. The values of R and SEC were 0.994 and 0.48 gL^{-1}, respectively. As the results of the validation of the calibration equation, Equation 2, SEP was 0.45 gL^{-1}. Excellent agreement between C_{act} and C_{pre} was observed with $r = 0.994$.

Monitoring of MEL and soya oil concentrations in the cultivation

Monitoring of the MEL and soya oil concentrations in the culture broth during the glycolipid fermentation was performed using the calibration equations, Equations 1 and 2. Time courses of the concentrations of MEL, soya oil and microbial cells during the fermentation were shown in Figure 3. As the microorganism growing, MEL was produced to take soya oil into the microorganism. The values of the concentrations of MEL and soya oil measured by the NIR method were in good agreement with those obtained by the conventional method.

Discussions

The TLC/FID method was applied to the measurement of the concentrations of MEL and soya oil in the ethyl acetate extract of the glycolipid fermentation. Although the operational procedure of the TLC/FID method is simple, the time required for the measurement is about 50 min per sample in addition to 30 min for the extraction procedure. However, the operational procedure of NIR was also very simple and the time required for the measurement was only 5 min in addition to 30 min for the extrac-

tion procedure. The results of the present study suggest that NIR may be a useful method for the monitoring of the glycolipid fermentation.

Acknowledgment

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